THE DEVELOPMENT OF THE TYMPANO-EUSTACHIAN PASSAGE AND ASSOCIATED STRUCTURES IN THE COMMON TOAD (BUFO LENTIGINOSUS).

BY HENRY FOX.

A perusal of the literature relating to the subject reveals the existence of considerable diversity of opinion among investigators as to the exact morphological significance of the tympano-Eustachian passage of the higher vertebrates. So far as its adult structure and relations are concerned, the passage would seem to be the homologue of the spiracle or hyomandibular cleft of the elasmobranch fishes. Both structures occupy the same relative position between the mandibular and hyoid arches, and, moreover, above the dorsal margin of each the facial nerve divides into its two main branches, one of which, the ramus palatinus, courses in front of the cleft (or tube, as in the higher forms), while the other, the ramus hyomandibularis, extends ventrally along its posterior wall. Embryologists, however, in studying the development of the tympano-Eustachian passage in various species of the higher vertebrates, have found that its homology with the hyomandibular cleft is not so clearly expressed as the mature structure of the organ would lead one to infer, so that certain morphologists, basing their conclusions on the facts revealed by embryology, hold that the tympano-Eustachian passage is a structure entirely, or in large part, independent of the hyomandibular cleft.

In order to determine, if possible, the exact relation of the tympano-Eustachian passage to the hyomandibular cleft, I undertook to follow out its entire embryonic history in the common toad of the eastern United States, *Bufo lentiginosus*.[1] Contributions to the knowledge of the development of the structures under consideration had been made in the case of the *Anura* by Goette,[2]

---

[1] The investigations have been made in the Zoological Laboratory of the University of Pennsylvania.

[2] *Entwicklungsgeschichte der Unke, Bombinator igneus*, Leipzig, 1875.

Villy,[3] and Gaupp.[4] Shortly after I had begun the present
research a very important paper on the subject by Dr. Hans
Spemann appeared, treating of the earlier stages in the develop-
ment of the Eustachian tube in *Rana temporaria*.[5] All the inves-
tigators mentioned state that the development of the tympano-
Eustachian passage in the forms studied is a very indirect one and
that it can be traced only with considerable difficulty. This diffi-
culty is attributed to the almost complete atrophy of the hyoman-
dibular cleft, which at an early period becomes so greatly reduced
as to be readily overlooked unless special attention is bestowed
on it.

Of the investigators mentioned Goette correctly described the
degeneration of the hyomandibular cleft, but his other results con-
cerning the development of the Eustachian tube may be disre-
garded, since his investigations were conducted at a time when less
favorable methods were at his disposal than we have at present.
From the results arrived at by the other three investigators a fairly
complete history of the Eustachian tube may be made out in the
case of *Rana temporaria*. Of these the work of Villy covers
fairly well the period of the metamorphosis, although his descrip-
tions are somewhat inexact,[6] and his conclusion, that the Eusta-
chian tube " has almost certainly nothing to do with the hyoman-
dibular cleft," and that " the evidence offered by the frog tends to
show that the two organs have no connection whatever with each
other," is certainly unsound, since such a connection between the
two has been established by the very careful work of Spemann on
the earlier stages of the tube in the same species. The correctness
of Spemann's conclusions are corroborated by the results which I
have obtained in *Bufo*. Gaupp's chief contribution consists in his
calling attention to the appearance of the tubal *Anlage* at a stage
earlier than that in which it was first observed by Villy. For
further information concerning the results arrived at by these
investigators the reader is referred to the papers mentioned.

---

[3] "The Development of the Ear and Accessory Organs in the Common
Frog, Rana temporaria," *Quar. Jour. of Micros. Sci.*, 1890.

[4] "Beiträge zur Morphologie des Schädels, I, Primordial-cranium von
Rana fusca," *Morph. Arb.*, V, 2, 1893.

[5] Spemann, "Ueber die erste Entwicklung der Tuba Eustachii und des
Kopfskelets von Rana temporaria," *Zoologische Jahrbücher*, 1898.

[6] As, for instance, he speaks of the tube as extending forward beneath the
palato-pterygoid bar, which it never does, but, instead, passes beneath the
quadrate. Moreover, his figures show it in the latter position.

I. Observations and Results.

I now turn to the description of the development of the tympano-Eustachian passage in the common toad. In this undertaking I shall first treat in detail the condition and relations of the structures under consideration in the different stages, beginning with the earliest, and then at the end of the paper summarize the chief features of this development.

Stage I (Pl. VI, fig. 1).—I begin at a stage when the hitherto almost spherical embryo has elongated and when the tail has grown out as a short stump. No external gills are as yet apparent. The head has become differentiated from the body proper and the region immediately posterior to it is marked by two or three slight dorsi-ventral grooves, indicating the position of the future branchial-clefts.

Pl. VI, fig. 1 is a coronal section of the anterior portion of an embryo of this stage. The section is slightly oblique, the right side being cut at a higher plane than the left. In this figure one will notice that the anterior extremity of the pharynx is still separated from the exterior, the conjoined endoderm and ectoderm forming at this point a solid partition of cells—the stomatodeal plate (*st.*). From this region posteriorly the cavity of the pharynx gradually widens out until it forms a spacious chamber, the sides of which are marked by four dorso-ventral grooves, marking the inner openings of the visceral-clefts. Just back of the fourth visceral-cleft the cavity narrows very suddenly to form the lumen of the œsophagus.

As shown by the figure, there are only four visceral-clefts (*Hym.*, 2–4 *v.f.*) marked out at the present stage. With the exception of the fourth, each of the clefts extends outward as a solid, double-layered plate of endoderm, continuous at its inner end with the epithelial lining of the pharynx and externally in contact with the deeper layer of the ectoderm. Only the medial portion of each cleft shows a lumen. The fourth visceral-cleft resembles the others, except that it does not as yet quite reach the epiblast.

---

[6] In the drawing the distal extremities of the clefts are shown separated by a narrow, clear area from this layer, but this condition, I think, must have been produced by shrinkage, a supposition which receives support from the rough and irregular character of the distal edge.

15

Between the visceral-clefts intervene the visceral-arches. The interior of each arch is made up of a mass of rather compact mesenchyme, consisting of scattered cells, containing numerous large yolk-spheres, barely distinguishable from those occurring in the endodermic lining of the pharynx. From this circumstance the limits of the endoderm are somewhat difficult to define clearly, and accordingly considerable care had to be taken in outlining it. The endoderm is, however, much more densely crowded with yolk-spheres and hence appears as a darker layer more or less clearly marked off from the surrounding lighter mesenchyme. Four visceral-arches are clearly differentiated, the two anterior of which are the mandibular $(k.m.)$ and hyoid $(h.m.)$ arches, while the other two are the first and second branchial-arches. In the former two a somewhat dense patch of mesenchyme can be seen occupying the centre of each. These patches are the *Anlagen* of the future muscles of these arches $(k.m.$ and $h.m.)$.

An examination of the remaining sections of the series to which fig. 1 belongs, shows that the pharyngeal cavity retains approximately the same size throughout its entire dorso-ventral extent and that throughout their entire length the visceral-clefts have about the same direction and relations as shown in the figure. Hence we may look upon the clefts as being solid folds of endoderm, compressed antero-posteriorly and elongated dorso-ventrally. Throughout their entire extent the first three clefts are apparently in contact with the deeper layer of the ectoderm.

The first or hyomandibular cleft resembles the other clefts in all essential respects, except that it extends slightly forward whereas the second extends transversely outward, while the remaining two course obliquely backward. A section of the cleft in almost any coronal plane presents the condition shown in the figure. Immediately dorsal to the outer extremity of the cleft the distal portion of the facial ganglion becomes continuous with the deeper or sensory layer of the ectoderm.

Stage II (Pls. VI, VII, figs. 2–7).—In this stage all five visceral-clefts are present, none of which opens to the exterior. The mouth is still separated from the pharynx by the stomatodeal plate. The external gills have budded forth as two minute, blunt, undivided processes from the sides of the first and second visceral-arches.

In specimens of the present stage the *Anlagen* of the various structures have so far differentiated that they are in most cases readily recognizable. The mesenchyme is less compact than hitherto. The *Anlagen* of the muscles are particularly well marked out as prominent patches of densely aggregated mesenchyme cells, containing numerous yolk-spherules. The bloodvessels also have begun to form in the head region.

Pl. VI, figs. 2, 3 and 4 are coronal sections of a tadpole of this stage. Of these fig. 2 was taken at a plane a slight distance above the floor of the pharynx. Comparing it with fig. 1 we find that anteriorly the stomatodeal invagination (*st.*) has deepened very considerably, although as yet not communicating with the pharyngeal cavity. The latter has much the same form as in fig. 1, except that posteriorly an additional visceral-cleft is present. Of these clefts the most anterior, the hyomandibular (*Hym.*), can be seen as a narrow, solid diverticulum of the pharyngeal wall, extending outward and terminating bluntly in the mesenchyme a short distance below the external ectoderm. All the remaining cleft outgrowths reach to and blend with the external ectoderm, although as yet not opening to the exterior. Within the body of each of the two anterior visceral-arches— *i.e.*, mandibular and hyoid—the muscles can be made out as irregularly defined patches of denser mesenchyme. That in the mandibular arch is the *Anlage* of the muscles of mastication (*k.m.*), while that in the hyoid arch is the *Anlage* of the depressor mandibulæ + depressor ossis hyoidei [7] (*h.m.*). Anterior to the first cleft is a small vessel, the mandibular aortic arch (*m.a.*), while on the left of the figure another vessel is to be seen posterior to the cleft. The latter is the hyoidean aortic arch (*h.a.*).

Pl. VI, fig. 3 is taken at a considerably higher level. On the right side we have passed above the dorsal margins of the visceral-clefts, so that the latter are shown only on the left side. This section passes in a plane approximately on a level with the base of the brain, the small dark patch in the median line in front of the pharyngeal cavity being the floor of the infundibulum (*inf.*). The hyomandibular fold can be seen extending outward and slightly forward. It will also be noticed that its distal[8] end approaches the

---

[7] Spemann includes these two muscles under the term "orbito-hyoideus."
[8] A word of explanation is necessary concerning my use of the terms "distal" and "proximal." Ordinarily these terms are used only in connection

skin more closely than in fig. 2. In fig. 4 we see the fold at its dorsal origin from the pharyngeal wall (*Hym.*). Here it is to be seen as a rather wide, shallow, blunt diverticulum of the latter. In the sections intervening between this and fig. 3 the distal end progressively moves peripherally as we pass down until it comes to occupy the position shown in the latter figure. Hence the dorsal edge of the cleft is higher in its proximal portion than in its distal part. In fig. 4 it will also be noticed that the proximal portion of the cleft in its dorsal portion approaches very closely to the origin of the second visceral-cleft. In the other two figures the cleft is separated throughout by a considerable interval from the second cleft. It follows from this that as it descends the plane of the first cleft moves forward also.

Grouping the facts so far obtained we find the hyomandibular cleft as a solid, two-layered diverticulum of the pharyngeal wall, which extends outward and somewhat forward to a point a short distance removed from the external ectoderm. Here it terminates in a blunt, rounded edge, extending downward and slightly forward and presenting throughout its course no well-marked indentations or depressions. Above and below, however, the outer edge gradually recedes more and more from the skin until it blends imperceptibly with the lining of the pharyngeal cavity. The edge thus has the form of a gentle arch. In general the cleft outgrowth is elongated dorso-ventrally, but it also is directed obliquely forward. This forward direction is more pronounced in its dorsal than in its ventral portion. In its lower portion the cleft is widely separated from the second visceral-cleft, but in its dorso-posterior portion it approaches the latter very closely, particularly in its proximal, internal part.

These observations are further confirmed by transverse sections (Pls. VI, VII, figs. 5-7). In fig. 5 the hyomandibular cleft can be seen as a short, blunt diverticulum from the inferior, outer angle of the pharynx (*Hym.*). The cleft here is cut through its antero-

with processes or appendages of the body. In the present paper, however, I designate by "distal" that portion of the hyomandibular fold (or of its derivative, the Eustachian cord) which is farthest removed from its connection with the pharynx, while I employ the term "proximal" to denote that part of the same structure which is nearest the point of origin from the pharynx. My use of these terms in connection with the structure mentioned is due to the necessity of having some fixed term to apply to each of its extremities, the relative position of which vary in the different stages.

ventral portion. In the fifth section posterior to this (fig. 6) the cleft is cut throughout the greater part of its dorso-ventral extent, and hence appears as a broad, solid mass extending out from the side of the pharynx and reaching nearly to the skin, where it all but meets a slight papilla projecting inward from the latter (*Hym.*). This figure also reveals another feature of the cleft-outgrowth which is of particular importance. It will be noted that it is the upper portion of the cleft-fold which approaches most nearly the skin, whereas the ventral portion recedes gradually from it as we descend. About the middle of this ventral portion is a small indentation in the outer edge occupied by a small blood-vessel (*x.*). It will be also noticed that the proximal (inner) portion of the cleft is situated at a higher level than in fig. 5. From this it follows that the line of origin of the fold from the pharynx extends from below upward and backward.

Posterior to this region the hyomandibular fold bends more sharply backward and accordingly in transverse section appears considerably narrower (fig. 7, *Hym.*, right side). We next obtain the condition shown in fig. 5 (left side), where the fold (*Hym.*) is cut approximately at right angles to its surface and hence appears extremely narrow. Below the fold is a large oval mass, the *Anlage* of the hyoidean muscles, *i.e.*, depressor mandibulæ and depressor ossis hyoidei (*h.m.*). Spemann has noticed a relation between the subsequent development of these muscles and the degeneration of the hyomandibular fold. I have found the same relation to exist in *Bufo*, but shall call attention to it later. The fold next enlarges somewhat, and then, gradually receding more and more from the exterior, blends imperceptibly with the pharyngeal wall. These stages are shown consecutively in figs. 6 and 7 (left sides).

The transverse sections also show some structural features, which are of importance in tracing certain stages in the subsequent history of the cleft-fold. Anterior to the cleft is the efferent mandibular aortic artery, a branch from the carotid. At the present stage this vessel is rather difficult to trace, but with some care can be worked out. Since, owing to the general antero-ventral direction taken by the plane of the hyomandibular fold, the anterior wall of the latter faces forward and also upward, it follows that in transverse section structures anterior to the fold will be seen dorsal

to it. Thus in the figures the region immediately dorsal to the fold is the mandibular arch, whereas that ventral to it is the hyoid arch. In fig. 7 the efferent portion of the mandibular aortic arch can be seen as a transversely placed vessel (*m.a'.*) just above the roof of the pharynx and extending outward above the hyomandibular diverticulum. Internally the vessel unites with the carotid (*car.*). The course of the mandibular aortic arch can be followed by comparing the figures. At first it is very small, as seen in fig. 5 (*m.a'.*). Tracing it forward, however, it is soon found to be continuous with a much larger vessel with a well-marked lumen. This vessel is the afferent portion of the mandibular aortic arch (*m.a''*). Immediately beneath the antero-inferior extremity of the hyomandibular fold the mandibular aortic arch is joined by the hyoidean aortic arch, and the common trunk thus formed communicates with the large inferior jugular sinuses beneath the mouth.

The other structure to which I desire to call attention is the hyomandibular ramus of the facial nerve. The facial ganglion at present lies just back of and above the dorsal margin of the hyomandibular fold. The anterior edge of the ganglion is in actual contact with the outer margin of the fold (fig. 7, *vii*). From the ventral surface of the ganglion the hyomandibular ramus (fig. 6, *vii h.*) is given off as a large nerve supplying the muscles of the hyoid arch. It is hence posterior to the hyomandibular fold.

Stage III (**Pls.** VII, VIII, figs. 10–14, 16–18).—Young tadpole. External gills prominent and considerably branched, not covered as yet to any marked extent by the opercular fold. Third visceral-cleft opening to the exterior. Mouth communicating with pharynx. The tail has attained its full development.

A considerable departure from the conditions observed in the preceding stage is shown in the present. The different organs are quite clearly differentiated, while the *Anlagen* of the more important cartilages can be made out as dense aggregations of the mesenchyme. The first visceral-cleft especially has undergone marked modifications. We can follow out its course by comparing figs. 10–14. Consulting fig. 10, we notice that the pharyngeal wall is separated from the exterior by a considerable interval occupied by scattered mesenchyme cells, which in the region immediately surrounding the pharynx are segregating to form the *Anlagen* of the

skeletal structures. Since it will be necessary hereafter in study-
ing the development of the Eustachian tube to take into consider-
ation the modifications undergone by the neighboring skeletal
parts, it may be well to point out these parts in the present stage.
The very dense segregated mass which may be seen in fig. 10
(*M.* and *Q.*), immediately external to and beneath the pharynx, is
the *Anlage* of the cartilaginous mandibular arch. That portion
of the arch which underlies the pharynx is the mandibular or
Meckel's cartilage (*M.*), while that external to it is the quadrate
or suspensorium (*Q.*). In the figure there is no distinct separation
between these two portions, but more anteriorly the mandibular
Anlage can be seen to be separated from the quadrate by a slight
space in which the mesenchyme cells are less densely aggregated
(fig. 11, *M.*—shown here owing to the oblique section, the left
side being cut more anteriorly than the right). In fig. 10 (*M.*)
only the most posterior part of the mandible can be seen. The
mandible, as in all anuran tadpoles, extends transversely beneath
the floor of the mouth. External to the lateral wall of the
pharynx (right side) is the quadrate cartilage (*Q.*), which ven-
trally becomes continuous with the mandible and at the same point
sends upward and outward a strong process, the orbital process or
processus muscularis (*Pr.M.*) (Gaupp). This process with the
inner portion of the quadrate forms a deep concavity, underlying
the eye and containing the muscles of mastication. That portion
of the quadrate which lies in contact with the pharyngeal wall is
the palato-pterygoid process or commissura quadrato-cranialis ante-
rior of Gaupp (fig. 11, *Pr.q.c.a.*). At its dorsal extremity this
part approaches, but is still separated from, a patch of dense tissue
in immediate contact with the dorso-lateral border of the pharynx,
the *Anlage* of the trabecula cranii (*Tr.*).

On the right side of fig. 11 (fourth section posterior to that of
fig. 10), the mandibular cartilage has been passed, and in its stead
we find a very slight aggregation of mesenchyme forming a portion
of the cartilaginous hyoid bar. The trabecula cranii of the same
side has become much less distinct, and in the third section follow-
ing (fig. 12) has ceased to be any longer distinguishable from the
surrounding mesenchyme. The trabeculæ cranii at present are
thus marked out only in their more anterior portion. Of the
quadrate cartilage we have only the body with its processus mus-

cularis, having passed beyond the transversely placed commissure quadrato-cranialis anterior (palato-pterygoid). Of the quadrate the outer, distal portion of the processus muscularis is most distinct at the present stage. Above this process are the muscles of mastication (*k.m.*) already mentioned, while to its outer or ventral surface are attached two muscles, the depressor mandibulæ (*m.d.m.*) and depressor ossis hyoidei (*m.d.h.*). Between these two muscles courses the ramus hyomandibularis of the facial nerve (*vii h.*). Both of these muscles belong to the hyoid or second visceral-arch and have been differentiated out of the common muscle mass of that arch.

The quadrate in the region posterior to that just considered blends gradually and imperceptibly with the surrounding mesenchyme. This can be followed by examining the figures consecutively.

We will now turn to the consideration of the hyomandibular fold in the present stage. In fig. 12 the rhomboidal cavity of the pharynx is sharply prolonged at its right ventro-lateral angle, and from the wall of the cavity immediately above this prolongation a narrow, solid cord, representing an extension of the wall, extends upward and outward in close contact with the ventral surface of the processus muscularis (*Eu.*). Just internal to its blind, distal extremity can be seen a small vessel interposed between the cord and the cartilage. This vessel is the mandibular aortic arch. Ventral to the cord is a semicircular mass of procartilage, in the hollow of which is placed the depressor mandibulæ. This is the *Anlage* of the hyoid, a more complete view of which can be obtained in fig. 13 (*H.*). The hyoid, like the mandible, is a stout, thick bar placed transversely beneath the floor of the pharynx and separated from its fellow in the mid-line by a less compact tissue. Anteriorly the two are separated by the thyroid gland outgrowth (*Th.*). At its outer extremity the hyoid turns sharply upward as a flattened plate with a concave outer surface in which is lodged, as already mentioned, the depressor mandibulæ. Its inner surface is closely applied to the outer and ventral wall of the hyomandibular fold (*Eu.*).

In the region posterior to that shown in fig. 12 the hyomandibular fold presents much the same appearance as in the last stage (compare figs. 13 and 14 with 6 and 7). It will be noticed, how-

ever, that the fold is considerably narrower than in the preceding
stage, and also that its distal extremity is much farther removed
from the external surface.   This condition will be more fully con-
sidered presently.   The narrowing of the fold, however, is more
apparent than real.   If one will bear in mind the statement
already made that the fold extends downward and obliquely for-
ward, a true explanation of the difference will suggest itself.
Naturally a section which passes through in the same plane as that
of the fold will show the latter as a broad mass.   This explains
the appearance of the fold as shown in fig. 6 (right side).   In this
figure the section on the right side passes through the eye, whereas
on the left side it passes some distance behind the eye.   Hence the
section traverses the right side in an obliquely forward direction,
thus coinciding in the main with the plane of the fold.   In the
same specimen the fold on the left side is cut throughout trans-
versely, so that, except in its most posterior portion, it appears as
a narrow, two-layered lamina.

It is in its distal anterior portion that the hyomandibular fold
has undergone its greatest modification.   In fig. 12 the fold is
continuous with the wall of the pharynx.   In fig. 11, which is the
third section anterior to that of fig. 12, this connection no longer
exists.   The fold appears as a solid, somewhat flattened cord
(*Eu.*), closely underlying the upper, outer extremity of the pro-
cessus muscularis.   Its internal surface is in intimate contact with
the mandibular aortic arch (*m.a.*), while externally the two muscles
of the hyoid arch—*i.e.*, depressor mandibulæ (*m.d.m.*) and depres-
sor ossis hyoidei (*m.d.h.*)—approach it very closely.   The proxi-
mal portion of the anterior part of the fold can be seen in the
figure as a relatively broad diverticulum from the wall of the
outer, inferior angle of the pharynx (*Hym.*).

Anterior to the region just considered this cord-like extension of
the fold extends forward a short distance and then bends sharply
outward in front of the two muscles just mentioned (fig. 18, *Hym.*,
right side).   In this region it enlarges considerably and finally
terminates as a blind, bulbous swelling in the mesenchyme a short
distance below the external epithelium.   This part is shown in fig.
10 (*Tym.*), also in fig. 17 (*Tym.*).

Perhaps a clearer conception of the state of the fold may be
gained by a comparison with some coronal sections.   In fig. 16 we

have such a section, in which, however, the plane is lower on the right side than on the left. Commencing below, we observe on the right of the figure a short, blunt diverticulum of the pharyngeal wall, extending outward and slightly forward between the *Anlagen* of the mandibular and hyoid cartilages. This part corresponds to broad proximal portion of the fold shown in fig. 11 as continuous with the pharyngeal wall. In the fourth section dorsal to this (fig. 17) the same portion of the fold is still seen, and just external to its distal extremity is an elongated strand of like nature (*Tym.*), somewhat swollen in its outer portion, where it terminates just beneath the external epithelium. This part is the swollen portion of the cleft, which, as already mentioned, extends out in front of the hyoidean muscles and forms the distal expanded portion of the cord-like extension of the fold. In the second section above this (fig. 18) these two parts of the fold join, so that it now appears continuous throughout (*Hym.*). The present section gives a very good view of the course taken by the hyomandibular fold. One will observe that it has a very broad origin from the pharyngeal wall, and that from this point it extends outward and also considerably forward. In its middle portion the fold is considerably constricted, while in its distal outer extremity it is enlarged to form the swollen, bulbous portion which curves outward in front of the hyoidean muscles, as is well shown in the figure.

One notices that in fig. 18 the outer, distal extremity of the fold is farther removed from the exterior than in fig. 17. If the left side of fig. 16 (*Hym.*)—which represents a plane slightly more dorsal than that of the right of fig. 18—be now consulted it will be seen that this portion is still farther removed from the exterior, and by comparing the same fold (*Hym.*) in the following two figures (17 and 18) the distance between the two will be seen to be still more increased. In the latter two figures the fold approaches very closely the proximal portion of the second visceral-cleft (*2 v.f.*)—a feature to which we have already called attention.

Bringing together the facts so far obtained relating to the third stage, we shall now endeavor to form a conception of the hyomandibular fold as a whole. It arises as a solid fold of the wall of the pharynx and extends downward and obliquely forward as a thin plate between the first and second visceral-arches. Its origin from the pharynx extends downward and forward, beginning above

just anterior to the dorsal origin of the second visceral-cleft (figs. 14, 17, 18, *Hym.*) and terminating at the position of the future quadrato-mandibular articulation (fig. 10, *Hym.*). The outer or distal border begins dorsally in continuity with the roof of the pharynx (fig. 14, *Hym.*), and then extends in a gentle curve downward, outward and forward until it reaches the point where the distal, cord-like extension is given off and which I shall now designate as the "diverticulum." The latter is at first a flattened cord (fig. 11, *Eu.*), which at first extends forward a short distance, but, when it reaches the anterior border of the depressor ossis hyoidei, turns sharply outward and slightly downward in front of the latter and then expands to form a solid, bulbous swelling, which terminates blindly in the mesenchyme a short distance below the external ectoderm (figs. 10, 17, *Tym.;* also fig. 18, *Hym.*). This portion of the hyomandibular fold is the only part which comes into close proximity with the external epithelium. The remainder of the fold lies at a considerably deeper level. The distal border of the latter, below the origin of the "diverticulum," bends downward and inward and at its ventral end blends with the floor of the pharynx (figs. 10, 11, *Hym.;* 12, 13, 14, *Hym.* [left side]; 16, 17 [right]). This portion of the distal border is continuous with the ventral border of the "diverticulum," and, owing to the slightly downward direction taken by the latter, forms with it a shallow sinus or depression, the concavity of which faces downward and outward. By its anterior surface the hyomandibular fold is in close contact with the quadrate, although partly separated from it by the mandibular aortic arch (*m.a.*). Owing to the obliquely anterior direction taken by the hyomandibular fold, this surface faces both forward and upward, so that in transverse sections it appears as the dorsal border. Hence it follows that all structures found above the fold are anterior to it, whereas those ventral to it are posterior. The posterior surface faces backward and downward and has in close relation the *Anlagen* of the hyoid cartilage and associated muscles. Between the two muscles is the ramus hyomandibularis of the facial nerve (*vii h.*) which occupies its definitive position posterior to the hyomandibular fold.[9]

[9] The reader will do well to consult figure 3 of Dr. Spemann's paper, which shows a reconstruction of the hyomandibular fold of *Rana temporaria* at a similar stage. I find that the fold in *Bufo lentiginosus* is in all essential respects similar.

It now remains for us to point out the differences between the
hyomandibular fold in the present and preceding stages and, if
possible, to ascertain how such differences have been produced.
In the first place, one will recall that the outer border of the fold
in the last stage described a gentle curve, arching from above
downward and forward, and that throughout the greater part of its
length this border approached very closely the external epiblast.
In the present stage the arch described by the outer border is
interrupted about its middle by a club-shaped " diverticulum,"
which, again, is the only portion of the fold which approaches
closely the external epithelium. The remainder of the outer bor-
der lies a considerable distance below the skin. Again, a compari-
son of coronal sections shows that the anterior extension of the
fold is more marked than in the earlier stage. Hence there are
at least three differences to be accounted for, *i.e.*, (1) the recession
of the outer border of the fold from the external epithelium; (2)
the formation of the blind, distal " diverticulum," which still
retains the original position of the fold near the skin, and (3)
the more anterior direction taken by the fold.

In order to account for these changes it is evident that at least
two factors must be borne in mind. These are (1) the growth
process—*i.e.*, the general increase in size of the parts in accordance
with the growth of the individual—and (2) the differentiation of
new structures. First, as regards the recession of the outer border
of the fold from the skin : By comparing the figures illustrating
the two stages, one will observe that a considerable increase in the
transverse diameter of the head has taken place, whereas little, if
any, increase has occurred in the vertical plane. On the other
hand the pharynx has not increased concomitantly in size, but,
instead, has undergone an actual decrease, so that it is not only
relatively, but also absolutely, smaller in size than in the earlier
stage. However, between the points of origin of the hyoman-
dibular fold the pharyngeal cavity retains approximately its orig-
inal width, a feature due to the fact that in this region it forms a
pair of shallow evaginations (fig. 16). The smaller size of the
pharyngeal cavity can be readily made out in the transverse sec-
tions (compare figs. 5–7 with 10–14). This reduction is in all
probability connected with the increase in amount of mesenchyme
and particularly with the segregation of the latter to form the *Anla-*

gen of the cartilages and muscles. The cartilages are laid down
close to the wall of the pharynx, and with their increase in size the
latter is naturally reduced; while at the same time the increase in
amount of the general mesenchyme accounts for the increase in
width of the entire head.

As a consequence of the retarded growth of the pharynx and of
the increase in width of the head it follows that, unless there is
sufficient rapidity of growth in the fold to compensate for the
arrested growth of the pharynx, the distal extremity of the
hyomandibular fold will be removed more and more from the exte-
rior and that ultimately it will come to lie quite deeply. To such
causes, I think, must be attributed the recession of the hyoman-
dibular fold from the exterior. The head has increased in width,
while the pharynx has remained stationary, and even been reduced
in size, so that its appendage, the fold, quite naturally recedes from
the ectoderm.

But this explanation suggests another problem: Why does the
fold not exhibit sufficient rapidity of growth to enable it to retain
throughout its original position near the external epithelium, as in
the case of the remaining visceral-clefts, and, moreover, why does
it retain this position at one point, *i.e.*, where the blind, bulbous
" diverticulum " terminates ? This question brings us to our
second topic—the formation of the " diverticulum." This part is
not, I consider, a new formation, but merely that portion of the
fold which has managed by its normal growth to retain its original
position near the ectoderm. In this connection I wish again to
call attention to the condition in stage II. The distal border then
formed a gentle arch, which for a considerable part of its extent
was in close proximity to the skin. However, at both its dorsal
and ventral extremities this border recedes progressively more and
more from the exterior until finally it blends at both ends with
the lining of the pharynx. Hence in sections the dorsal and
ventral portions of the distal border are seen at varying levels
below the ectoderm, while the crown (of the arched plate) is situ-
ated near the latter (compare figs. 2–7). Fig. 6 is instructive in
this connection. Take the fold as shown on the right side. It will
be seen that the distal edge is in close proximity to the ectoderm
for a considerable part of its length. However, the upper portion
of this border is closer to the ectoderm than the remainder. This

part represents a region slightly dorsal to the middle portion of the distal border. Below this the edge recedes to a slight extent from the exterior and in its middle portion forms a slight, barely perceptible depression. If we now conceive that in the future growth of the animal all the lower portion of the distal edge remains stationary and that the middle depression deepens considerably, while the upper portion alone remains in proximity to the ectoderm, then we should obtain a condition very similar to that shown in fig. 12, except that complete outward extension of the fold is not shown in the figure (see instead fig. 10). In fig. 12 the arrested ventral portion can be seen as an extension of the right inferior angle of the pharynx, while the concavity between it and the plate-like hyomandibular fold is the much-deepened depression (see also fig. 11). In the latter figure the lower portion of the fold can be seen as a blunt extension from the ventro-lateral wall of the pharynx, while the flattened, oval mass external to and above it is the dorsal portion, or, as we have temporarily termed it, the " diverticulum." More posteriorly, as shown in fig. 12, this " diverticulum " becomes continuous with the proximal portion of the fold, and accordingly the area embraced between these two portions anteriorly represents the depression, which we saw beginning in fig. 6. One will notice that in this area a muscle— the depressor mandibulæ ($m.d.m.$)—has just attained attachment to the *Anlage* of the quadrate cartilage, while external to it its companion muscle, the depressor ossis hyoidei ($m.d.h.$), has acquired attachment to the tip of the processus muscularis. The " diverticulum " lies between these two muscles and, as already mentioned, extends anteriorly between them until it reaches the anterior surface of the outer muscle (depressor ossis hyoidei), around which it curves outward (fig. 10, also 17 and 18). In the behavior of these two muscles lies the clue to the solution of the problem under consideration. One will recall that both of these muscles belong originally to the hyoid arch, and consequently their acquirement of attachment to the quadrate is a later affair. In stage II the original hyoidean muscle-mass, from which these two are subsequently differentiated, extends in its long axis almost vertically and is situated entirely behind the hyomandibular fold (see figs. 2–7). Later, however, as the muscle increases in size its long axis becomes extended in an obliquely anterior direction,

the superior border facing forward. At this time the common
muscle divides into an inner and anterior mass, the depressor man-
dibulæ, and an outer and posterior mass, the depressor ossis
hyoidei. With subsequent growth both muscles extend forward
more and more until one of them—the depressor mandibulæ—
invades the area intervening between the skin and the inferior por-
tion of the distal edge of the hyomandibular fold at the point
indicated by the slight depression shown in fig. 6 (x). Here its
anterior extremity comes into close relation with the segregating
Anlage of the quadrate at a point just in front of and below the
fold. The outer muscle—the depressor ossis hyoidei—also acquires
attachment to the quadrate *Anlage*, but at a point above and pos-
terior to the fold.

I have just mentioned that the depressor mandibulæ extends for-
ward in the space between the lower portion of the distal border
of the hyomandibular fold and the skin. With this invasion an
effective barrier is interposed between the two ; and as a result of
the subsequent increase in size of the muscle and of the extension
in width of the head, it follows that this lower portion of the
hyomandibular fold will be arrested in its growth and will conse-
quently come to be more and more removed from the exterior.
At the same time the segregation of the mesenchyme to form carti-
lage *Anlagen* interposes additional barriers to the outward growth of
the fold. Hence it is possible to understand why it is that the
lower part of the fold should lie so far beneath the ectoderm as
shown in the present stage (figs. 11, 16, 17). On the other hand,
the dorsal portion of the hyomandibular fold—*i.e.*, that which
forms the " diverticulum "—is situated above the depressor man-
dibulæ, so that the latter does not interfere with its normal growth
and as a result this portion of the fold still retains its proximity to
the skin. With the increase in width of the head it has been carried
outward with the skin. In its proximal portion, however, this
part also has been encroached upon by the developing depressor
mandibulæ, and as a result it presents the form of a long-drawn-
out cord, narrow and flattened in its proximal part and swollen in
its terminal part, where it is not encroached upon to any great
extent by the surrounding structures.

Along the dorsal edge of the fold no well-marked changes, so
far as I have been able to determine, seem to have taken place.

In the figures (particularly figs. 17 and 18 [left side]) one will notice that the distal extremity is removed some distance from the skin, but this appearance, I consider, is simply produced by the obliquely ventral direction taken by the dorsal border, as has been already described.

This brings us to our third problem, *i.e.*, the more pronounced anterior extension of the fold. This, I consider, is correlated with the growth anteriorly of the two hyoidean muscles. Naturally as these extend forward they carry the fold with them. As a result of this the posterior surface of the fold comes to face outward, and the anterior inward. Hence in transverse sections structures external to the fold are also morphologically posterior, whilst those internal to it are morphologically anterior (compare transverse with coronal sections of present stage).

Stage IV.—Young tadpole. Opercular fold well developed, ending freely posteriorly and with the ends of the external gills protruding beyond its posterior margin. The various tissues for the most part clearly differentiated. True cartilage developed in the mandibular and hyoid arches (Pl. VIII, figs. 15, 19; Pl. IX, figs. 23, 24).

Beginning anteriorly the distal, blind extremity of the " diverticulum " appears as a transversely extended cord of cells, somewhat expanded distally, lying in the loose mesenchyme some distance below the external epithelium (fig. 24, *Tym.*). This cord is clearly distinguished from the surrounding fibrous tissue by its greater density, which naturally causes it to stain more deeply, and also by the presence within its substance of yolk spherules and numerous pigment granules, similar to those found in the mucous membrane of the pharynx. In the present stage the yolk spherules, although still present, are much less numerous than in the earlier stages and they soon disappear altogether, so that the dark pigment becomes the distinguishing feature of the cord. The lower proximal portion of the hyomandibular fold can be seen in the figure as a shallow protrusion (*Hym.*) from the ventro-lateral angle of the pharynx (compare with figs. 10 and 11). In the region immediately posterior the proximal portion is practically blended with the wall of the pharynx (figs. 15 and 19). In fig. 19 (right side) it again becomes distinguishable and soon becomes continuous with the prominent diverticulum *Eu.* (left side of fig. 19).

I will now return to the " diverticulum " in order to trace its
further course. From its distal extremity the " diverticulum "
extends inward and slightly backward in close contact with the
anterior surface of the depressor ossis hyoidei (*m.d.h.*, fig. 24,
Tym.), and then ascending slightly to pass over a large vessel, the
mandibular aortic arch (*m.a.*), it comes into close relation with
the external surface of the processus muscularis of the quadrate.
As it progresses inward the cord gradually decreases in diameter, so
that when it reaches the quadrate it is reduced to about a half or
even a third of the diameter of its distal expanded portion.

After reaching the external surface of the quadrate the reduced
" diverticulum " turns sharply posteriorly at the inner edge of the
depressor ossis hyoidei as a minute, cylindrical cord, (figs. 23, 15,
19, *Eu.*). Here it is closely applied to the processus muscularis
of the quadrate. Below and internal to it is the mandibular
aortic arch (*m. a.*), while bounding it externally is a small, acces-
sory slip from the depressor mandibulæ (fig.15, *m.d.m'.*), the main
body of which is attached to the quadrate anterior and internal to
the cord (fig. 24, *m.d.m.*). The cord extends posteriorly in the
same position, usually closely applied to the quadrate, and showing
more or less reduction in size, so that in certain parts of its course
it is difficult to trace clearly. Throughout its entire extent, how-
ever, it contains numerous pigment granules, the presence of
which facilitates considerably the tracing of the cord, as does also
the scattered yolk-bodies apparent for the last time in the present
stage.

In fig. 15 the cord can be seen, much reduced, just under the
transversely extended processus muscularis and external to the
mandibular aortic arch (*m.a.*). In fig. 19 (right side) the cord
(*Eu.*) still occupies the same relative position. Just external to it
is the ramus hyomandibularis of the facial nerve (*vii h.*). Inter-
nal to it the mandibular artery (*m.a.*) intervenes between it and
the pharyngeal wall. At this point the mandibular aortic arch
begins to turn inward in order to reach the carotid. Immediately
behind the artery the cord fuses with the distal extremity of the
diverticulum extending up from the pharyngeal wall (see fig. 19,
Eu., left side). Here both the cord and proximal portion of the
hyomandibular fold become continuous. The fold becomes more
prominent in the following sections (fig. 23, *Eu.*) and ultimately

16

blends with the wall of the pharynx dorsal to the inner opening
of the first branchial-cleft.

To recapitulate briefly the state of the hyomandibular fold at
the present stage : we have found the ventral portion of the fold
present only as an inconspicuous protrusion of the ventro-lateral
angle of the pharynx. Only the dorsalmost portion of the orig-
inal fold is well developed, and from this the greatly prolonged
" diverticulum " extends forward as a solid cord of cells. The
latter originates posterior to the quadrato-hyoid articulation.
Throughout the greater part of its length the cord is closely applied
to the outer surface of the processus muscularis. Anteriorly,
however, it bends sharply outward in front of the depressor ossis
hyoidei and terminates blindly as a somewhat bulbous enlargement
in the subcutaneous tissue.

I may here describe briefly the condition of the neighboring
skeletal structures, since in the present stage these have acquired
the relations which they retain throughout the entire larval period.
The animal has now passed beyond the pro-cartilage stage and
consequently the cartilages can be readily traced. In most cases
they already show a well-defined perichondrium. The quadrate
cartilage is prolonged in an antero-posterior direction almost parallel
with the corresponding trabecula cranii. Its course is thus quite
the reverse of that which characterizes its adult condition. Its
distal articular end is prolonged as the processus articularis down-
ward and forward to a point beneath the anterior surface of the
eye and at a later period still farther forward. At its distal
extremity it bears the transversely placed mandibular cartilage
(Meckel's). The greater part of the quadrate is prolonged
upward and outward as a stout plate immediately underlying the
orbit—the processus muscularis—to the outer side of which are
attached the depressor mandibulæ and depressor ossis hyoidei. In
the concavity formed in the inner (and upper) surface are lodged
the muscles of mastication (fig. 24, $k.m.$). On the ventral surface
near the point of junction between the body of the cartilage and
the processus muscularis there is forming at the present stage a
shallow, concave articular surface for the head of the hyoid car-
tilage. The latter is a stout bar of cartilage extending trans-
versely beneath the floor of the pharynx and joined to its fellow
of the opposite side by the intervention of a median plate, the

copula. In its outer portion the hyoid turns sharply upward to form an ascending process, which articulates with the quadrate.

In its anterior portion—*i.e.*, where the processus articularis is given off—the quadrate is joined to the trabecula of the same side by an ascending bar of cartilage, the commissura quadrato-cranialis anterior (Gaupp) or palato-pterygoid bar. Posteriorly again the quadrate bends sharply upward and then as a stout bar (processus ascendens, fig. 19, *Pr.A.*) extends inward back of the eye and in front of the auditory capsule to join with the trabecula just in front of the basilar plate (parachordal). There is no distinct separation between these connected cartilages, the matrix of each being perfectly continuous with that of the others.

It now remains to connect the conditions observed in the present stage with those seen in the preceding. The chief differences between the former and the latter are briefly these: (1) The relatively much greater length of the "diverticulum," a condition associated with the removal of the part connecting it with the pharyngeal wall to a point more posterior, *i.e.*, back of the quadrato-hyoid articulation; (2) the reduction in size of the middle portion of the "diverticulum," and (3) the almost complete obliteration of the ventro-anterior portion of the hyomandibular fold.

These differences are, I believe, correlated with a continuation of the same processes treated of under the description of the preceding stage. These are chiefly the modifications undergone by the neighboring muscles and cartilages. The general growth of the animal has had little, if anything, to do in producing the differences between the two stages. There has been a considerable increase in width of the head—an increase in which, however, the contained structures have taken part. The greater length of the "diverticulum" has been produced by the continued increase in depth of the depression in the distal border of the fold. In stage III this depression was relatively shallow, so that the "diverticulum" was very short and blunt. In the present stage the "diverticulum" is very long, having the form of a long, narrow cord somewhat expanded at its distal extremity. The insinking of the distal border was associated with the growth of the depressor mandibulæ, in consequence of the latter's acquisition of a point of attachment to the quadrate in front of and below the distal border of the hyomandibular fold. In the present stage this muscle has

increased in size and extended its area of attachment to the quad-
rate. It has also given off an accessory slip, which extends
upward external to the cord-like " diverticulum " to attach to
the processus muscularis (fig. 15, $m.d.m'.$). Moreover, immedi-
ately behind the posterior edge of the depressor mandibulæ, the
hyoid cartilage is drawn up to form an articulation with the quad-
rate, and following this the enlarged mandibular aortic arch turns
inward to join with the carotid (fig. 19, $m.a.$) just in front of the
point where the " diverticulum " joins the extension from the
pharyngeal wall (fig. 19, $Eu.$, left side). Thus changes in three
structures have been instrumental in producing the deepening of
the depression, i.e., (1) the increase in size and area of attach-
ment of the depressor mandibulæ; (2) the articulation of the
hyoid to the quadrate, and (3) the increase in size of the man-
dibular aortic arch.

The reduction in size of the middle portion of the " diverticu-
lar " cord (compare figures with fig. 11 of last stage) has been
associated with two factors: (1) the increase in size of the accom-
panying mandibular aorta, and (2) the differentiation and growth
of the outer, accessory slip of the depressor mandibulæ. By
examining figure 15, one will notice the reduced cord tightly
wedged in between the enlarged artery internally and the accessory
slip externally.

The decrease of the ventro-anterior portion of the hyomandibular
fold to form a mere shallow protrusion of the ventro-lateral angle
of the pharynx (fig. 24, $Hym.$) has in all probability been pro-
duced by the deepening of the depression and its final blending
with the pharyngeal wall. Naturally, as the depression deepened
its deepest part would ultimately blend with the pharyngeal wall,
so as to be no longer distinguishable (fig. 15). As the ventro-
anterior portion of the fold formed the lower border of the depres-
sion, it would naturally be drawn in with the deepening of the
depression until it formed the shallow protrusion mentioned (fig.
24, $Hym.$). This decrease is also accelerated by the increase in
size and density of the skeletal and muscular parts.

Stage V.—Young tadpole of about 9 mm. Opercular cavity
communicating with the exterior by a single opening on the left
side. No external gills.

The condition of the hyomandibular fold is essentially similar to

that in the preceding stage.   The tissues of the animal are more
compact and definitely limited than in the last stage.   The
external, distal extremity of the " diverticulum " (or, as I may
now term it, the Eustachian cord, since the structure under con-
sideration ultimately gives rise to the greater part of the tube of
that name) has the same general appearance as before.   It, how-
ever, does not extend so far out from the processus muscularis as
before, a condition probably produced by the increase in size of
the process.   A slight reduction has also taken part in this portion
of the cord (" diverticulum ").   More marked, however, has been
the change in the middle portion of the cord.   After extending
inward to the processus muscularis the cord rapidly degenerates,
becoming greatly flattened and much reduced in size, so that for a
part of its course it is very difficult to recognize, the presence of
scattered nuclei and numerous pigment granules alone serving to
mark its existence.   This great reduction has been associated with
a continuation of the processes described in the last stage, i.e., the
growth of the hyoidean muscles (depressor mandibulæ and depres-
sor ossis hyoidei), the articulation of the hyoid with the quadrate
and the increase in size of the mandibular artery (Pl. VII, fig. 8
and Pl. IX, fig. 25, Eu.).

The cord retains the degenerate condition just described until it
reaches a point just back of the region where the mandibular aorta
turns inward to join the carotid.   A good idea of the condition of
the cord can be obtained from coronal sections (fig. 8).   In such
it appears as a faint, narrow cord (Eu.), coursing in an antero-
posterior direction in contact with the outer surface of the pro-
cessus muscularis.   This cord contains no lumen and shows no
indication of a tubal character.   It contains throughout its course
scattered nuclei arranged end to end, and it is largely colored by
numerous black pigment-granules.   The yolk-spherules have now
disappeared entirely.   There is very little substance to the cord
and in places where nuclei and pigment are lacking it becomes
very difficult to trace.

Immediately posterior to the inflexed mandibular aorta the
Eustachian cord is joined to the pharyngeal wall by a narrow
strand of somewhat elongated cells.   These cells are not easily
distinguishable from the cells of the surrounding connective tissue,
but they form a rather dense patch in the latter stretched between

the pharyngeal wall and the tubal *Anlage*. Immediately beyond this region this connecting portion broadens out considerably to form a rather shallow bulging of cells from the roof of a " lateral recess " (fig. 25, *l.r.*) or pouch of the pharynx, from the outer extremity of which the thymus gland is given off. This " lateral recess " is really formed by an extension outward of the branchial portion of the pharynx over the internal branchial openings, so that the latter are now situated on the floor of the cavity. In the preceding stage this " lateral recess " was just beginning to form as a slight bulging beneath the proximal portion of the tubal *Anlage*. With the extension externally of the " lateral recess " the proximal portion of the Eustachian cord comes to appear as an inconspicuous protuberance over the inner part of the roof of the " recess " (fig. 25, immediately internal to *vii h*).

Stage VI.—Tadpole of 18 mm. Posterior limbs appearing as minute buds below the root of the tail (Pl. IX, fig. 26).

In this stage the Eustachian cord has about reached the height of its degeneration. The cord still maintains the same general relations to the surrounding parts as before. Its distal, expanded extremity remains distinct, and from thence the cord can readily be traced to its characteristic position next to the outer surface of the processus muscularis. Here, however, it soon becomes very small and then can be traced only with the greatest difficulty. The cord lies immediately above the mandibular aorta, and by following the latter it may be traced as a minute, more or less flattened pigmented patch, which in certain parts contains one or two nuclei not readily distinguishable from the nuclei of the surrounding fibrous tissue (fig. 26, *Eu.*). Immediately posterior to the quadrato-hyoid articulation the cord again enlarges slightly and can be traced thence for a considerable distance. Then in the region where the ramus hyomandibularis begins to come into close relations with its external surface all distinct traces of the cord are lost. Nothing more of the cord is to be made out until we come to the region where the mandibular artery turns inward, where for a short space the cord is again revealed and then terminates without forming any clear connection with the pharyngeal wall. This most posterior fragment of the cord is situated below the quadrate, dorsal to the upper anterior end of the " lateral recess " of the pharynx. There is no distinct proliferation from the dorso-

internal wall of this portion to indicate the proximal, connecting part of the tubal cord. The proliferation has very likely opened out with the formation of the " lateral recess," and has been merged into the dorsal wall of the latter.

It thus appears that in the present stage the Eustachian cord for the greater part of its length has undergone remarkable fragmentation, having broken up into a number of sections of variable length. Each of these fragments, however, retains exactly the same relations to the surrounding structures that the corresponding part of the cord showed in the preceding stage. It is quite possible that the various fragments may still be connected by the transparent cell-walls of the cord, and in that case the apparent fragmentation is simply due to the restriction of the more vital, stainable portions to areas less subject to the action of unfavorable forces. I am somewhat inclined to consider this the actual condition in the present stage, since in a longitudinal series I have been enabled to follow out with great care a pale, almost transparent cord connecting some of the fragments. Posteriorly this cord approaches very closely the wall of the pharynx. I have not been able to make out any distinct connection between the two, but their proximity would incline me to believe such a union to exist. Still I have not been able to satisfy myself on this point.

I have not been able to determine to my satisfaction the factors which have been concerned in the degeneration of the Eustachian cord. One of them is probably to be found in the pressure exerted by the surrounding structures, particularly by the two muscles already mentioned. Owing probably to its unfavorable position the tubal cord appears to have little, if any, power of independent growth. It therefore may have been acted on by the growth anteriorly of the head whereby a pull has been exerted on it, causing its wall to extend and its contents to be restricted to more or less limited regions of the cord.

Stage VII.—Tadpole of 21 mm. Hind limbs well developed.

This stage very closely resembles the preceding. Owing to an accident the more anterior sections of the Eustachian cord in the specimen examined are lacking, but I have no doubt but that this portion of the cord in the present stage corresponds in all essential respects with that in the preceding, since in the succeeding stage the anterior portion is very similar to that in stage VI. So far

as the remaining parts are concerned, they present the same frag-
mentary character as in the preceding stage, being in certain loca-
tions almost unrecognizable. I noticed in the present series (and
likewise in several later ones) that there is no necessary corre-
spondence either in the number, length or distribution of the frag-
ments of the two sides. In the case of the specimen of the present
stage examined the sections were almost exactly transverse, so that
the same parts were cut on both sides. Yet the tubal cord may
be present for a considerable distance on one side and apparently
altogether absent on the other. This irregularity is a marked
feature during the entire metamorphic period. I find that there
is also marked individual variation in this respect. This vari-
ability would seem to indicate that the character of the fragmen-
tation is not due to some inherited tendency, but is produced by
mechanical forces exerted by the surrounding structures.

Posteriorly the Eustachian cord terminates suddenly in the usual
position, dorsal to the anterior extremity of the " lateral recess "
of the branchial portion of the pharynx. In the same region a
prominent proliferation arises from the dorsal wall of the " recess,"
and extends upward to the same relative position as that occupied
by the cord in the more anterior sections. This structure may
represent the same mass of cells which originally established the
connection between the tubal cord and the wall of the pharynx, but
of this interpretation I am uncertain, since I was unable to discover
any sign of such proliferation in the preceding stage or in a num-
ber of later stages. Possibly its occurrence or absence is a matter
of individual variation.

There has been but little change in the skeleton since the last
period. Posteriorly, however, the quadrate has developed a pos-
teriorly projecting processus oticus, which comes in contact with
the ventral surface of the auditory capsule. The processus oticus
arises at the angle formed by the body of the quadrate with the
processus ascendens. The stapes appears for the first time as an
oval chondrification within the membrane closing the fenestra
ovalis. There are no distinct traces of a columella auris.

Stage VIII.—Tadpole of 21 mm. Preceding the appearance of
the fore-limbs.

At this time we have the earliest distinct appearance of the annu-
lar cartilage (Pl. IX, fig. 28, *An.*). About opposite the point where

the pterygo-palatine bar (processus quadrato-cranialis anterior)
joins the quadrate, a very conspicuous proliferation from the peri-
chondrium of the latter occurs.   This proliferation forms a dense
strand of cells, which reach outward in the subcutaneous tissue
and aggregate themselves in a somewhat concentric fashion about
the distal, expanded termination of the Eustachian cord (*Tym.*).
The dense patch there formed is the *Anlage* of the future annu-
lar cartilage.   From this region the Eustachian cord pursues the
same course that characterized the preceding stages.   The cord
is, however, much more distinct than in any of the latter, and its
tubular character is plainly indicated by its nuclei, which are now
grouped about the periphery of the cord, thus giving the latter the
appearance of a duct with an obliterated lumen (Pl. XI, fig. 29,
Eu.).   In certain parts of the cord slight indications of a central
lumen can be made out, but, as a rule, any cavities that do appear
are neither very extensive nor pronounced.   The cord, however, as
in the preceding stages, becomes smaller as it extends posteriorly
and in the region of the quadrato-hyoid articulation disappears.   It
soon reappears, however.   Posterior to the hyoid articulation the
cord again becomes much reduced, but does not lose its continuity
with the most posterior portion.   In the most posterior part of its
course the cord again enlarges, becomes clearly tubular, and ex-
hibits a more or less well-defined lumen.   In this portion the cord
occupies its characteristic position, ventral to the quadrate cartilage
and internal to the ramus hyomandibularis, which in the region of
the quadrato-hyoid articulation ascends from the ventral portion of
the hyoid arch to come into close relation with the outer wall of
the cord.   From the dorsal wall of the " lateral recess " of the
pharynx a conspicuous strand of cells arises, the dorsal end of
which closely approaches the Eustachian cord, but before actual
contact takes place the cord rather suddenly terminates.   I am
not certain of the significance of this strand.   It may be the part
which originally connected the cord with the pharynx, but of this
I am uncertain, since I found no evidence of it in stage VI.

Stage IX.—Tadpole of 18 mm.   Both fore and hind limbs
present.

This stage marks the commencement of the metamorphosis.
Since the changes which the Eustachian cord (or tube) undergoes
during this period are obviously correlated with modifications

taking place simultaneously in the skeletal structures, it is neces-
sary, in order to follow the former, to obtain a right conception of
the latter. Hence in the present stage I will first treat of the
essential skeletal parts. In the first place, the axis of the quad-
rate extends in a more dorso-ventral direction than formerly, so
that now the mandibular articulation lies below the anterior edge
of the eye, instead of being entirely in front of it as before. This
position implies that the lower part of the quadrate has moved or
rotated backward through a slight angle. The processus quadrato-
cranialis anterior (pterygo-palatine) is now considerably elongated
in an antero-posterior direction, a change obviously associated with
the backward rotation of the quadrate. The processus muscularis
(orbital) begins to show signs of degeneration, especially along its
dorso-external edge. The hyoid still articulates to the ventral sur-
face of the quadrate. Posteriorly the processus ascendens has
degenerated and consequently the quadrate has lost its connection
with the wall of the brain-case, but instead it now joins by means
of its processus oticus the wall of the auditory capsule anterior to
the fenestra ovalis. In the membrane closing the latter the stapes
now appears as a large, oval mass of fully differentiated cartilage.
The columella auris is a delicate rod of primitive cartilage, closely
applied to the wall of the capsule. Anteriorly it terminates with-
out forming any connection whatever with either the quadrate or
any portion of the Eustachian tube and posteriorly it unites with
the stapes. The columella is most distinct and its tissue most
compact in its posterior portion, so that it cannot be regarded as
a derivative of the quadrate.

As a consequence of the posterior rotation of the distal portion
of the quadrate, the distal extremity of the Eustachian cord is
now situated somewhat posterior to its former position, but its
relation to the immediately surrounding structures is the same as
before, since these likewise have been affected by the quadrate's
change of axis. The annular cartilage, now a dense cellular mass,
is situated under the anterior margin of the eye and above the
mandibular articulation. To its ventral surface the more anterior
fibres of the depressor ossis hyoidei have acquired attachment.
Imbedded in the cartilage is the distal end of the Eustachian cord,
the future tympanic cavity. The general appearance of the cord
is similar to that in the preceding stage. The cord still shows

fragmentation, although the length and distribution of the frag-
ments differ on the two sides.   No connection between the cord
and the pharynx can be determined with certainty, although the
proliferation attached to the dorsal wall of the " lateral recess "
is still present.

Stage X.—Tailed toad of 15.5 mm.   Fore and hind limbs well
developed.

This stage very closely resembles the preceding, the most marked
differences being the greater antero-posterior elongation of the
processus quadrato-cranialis anterior and the associated greater
posterior rotation of the quadrate.   The Eustachian cord, also,
is very distinct, particularly in its anterior and posterior portions.
In the region just back of the quadrato-hyoid articulation it is
greatly reduced and traceable only with difficulty.   In several
places the cord shows a distinct lumen.   There are no distinct
signs of a proliferation attached to the pharyngeal wall extending
toward the cord.

Stage XI.—Tailed toad, 6.8 mm., tail 1.5 mm.   Close of the
metamorphosis.

The processus quadrato-cranialis anterior has now increased con-
siderably in length, so that it extends in a direct antero-posterior
direction as in the adult toad.   The axis of the quadrate has
attained an almost vertical direction, but it still extends somewhat
forward, its distal, articular end being located under the middle or
posterior part of the eye.   The hyoid still maintains its union with
the quadrate.   More posteriorly, in the region of the auditory cap-
sule, the columella auris can be traced farther forward.   At its
anterior, distal extremity it is prolonged forward as a dense strand
of cells, which forms a connection with the posterior surface of
the quadrate.   Only the more posterior portion of the columellar
rod is formed of true cartilage, the anterior portion being as yet
only a dense, undifferentiated mass of cells.

The most conspicuous changes which the tympano-Eustachian
tube has undergone since the preceding period have been asso-
ciated with the change of axis of the suspensory cartilages.   As a
result of this the tympanic portion of the tube, together with the
annular cartilage, has moved backward to a region below the
posterior portion of the eye.   The tube, as a whole, exhibits the
same fragmentary character as hitherto, and I have remarked here,

as in a number of other stages, a difference in the condition of the
tubes of the two sides. Posteriorly the tube terminates without
forming any connection with the pharynx. The proliferation
from the pharynx is not very distinct. It probably tends to dis-
appear in connection with the degeneration of the branchial appar-
atus.

Stage XII.—Tailed toad, 7 mm. Close of metamorphosis.

The quadrate cartilage now stands almost vertical, its distal,
articular extremity lying under the posterior border of the eye.
The hyoid bar has separated completely from the quadrate and its
dorsal extremity is now joined by the intervention of a dense
strand of cells to the base of the auditory capsule.

The distal part of the tympano-Eustachian tube now lies imme-
diately posterior and ventral to the eye. Since the distal portion
of the quadrate rotates backward more rapidly than the remaining
part, it results that the posterior, hitherto ventral, surface of the
cartilage forms a shallow concavity. From this behavior it follows
that the distal, expanded portion of the tube—*i.e.*, tympanic por-
tion—comes to lie farther posterior in relation to the rest of the
cord, so that the latter no longer presents an almost direct antero-
posterior course, but instead now lies in an almost transverse plane,
except for a slight anterior inclination. The tympanic region of
the tube is thus brought into relation with the auditory capsule.
In the present period it has not quite reached the region of the
latter, but is not far removed, being located just back of the eye.
Another feature shown by the present stage is the union of the
various fragments of which it was hitherto composed. This union
is also probably to be connected with the quadrate's change of
axis, since this would result in carrying the more anterior frag-
ments backward and thus bringing them into closer relation with
the posterior parts. At present the tube can be traced without a
break throughout its entire course. This fact speaks strongly for
the view that these parts have all along been united by an attenu-
ated cord. The fragments are simply the contents of this cord
which have been restricted to certain areas. As a result of the
backward rotation of the quadrate, the stretching to which the cord
had hitherto been subjected is relieved and accordingly the vari-
ous fragments of the substance flow together, thus producing the
union described. Proximally, however, the tube forms no con-

nection with the pharyngeal wall, but immediately internal to its
proximal termination the pharynx sends out a narrow cleft between
the hyoid and the base of the auditory capsule. Posteriorly the
tube ceases immediately in front of the dense strand connecting
the hyoid cornua with the auditory capsule, so that at this stage the
tube occupies its definitive position between the quadrate and hyoid
cartilages. As a result of the changes that have taken place in
the hyoid its nerve, the ramus hyomandibularis, now lies ventral
and posterior to the tube—a position which characterizes it in the
adult condition. In the branchial region the entire branchial
apparatus, including the " lateral recess " of the pharynx, has be-
come largely obliterated.

Stage XIII.—Young toad, 6 mm. Metamorphosis complete.

This period marks the close of the metamorphosis. The infe-
rior, articular portion of the quadrate extends more posterior, so
that the quadrate on its posterior surface shows a marked con-
cavity. The general course of the quadrate is about as follows:
Dorsally from its union with the base of the auditory capsule it
extends forward and downward for some little distance, it then
describes a wide curve downward and backward for the remainder
of its length, so that its distal end, bearing the mandibular car-
tilage, now comes to lie under or even slightly behind its dorsal,
proximal extremity. The hyoid arch is now fused completely with
the auditory capsule, the intervening cellular strand having become
cartilaginous.

The annular cartilage is now located posterior to the eye and
ventral to the anterior portion of the auditory capsule. It closely
underlies the skin and is external to the outer surface of the
quadrate. The Eustachian tube itself differs but little from its
condition in the preceding stage, except that its lumen, where
present, is more distinct and extensive. A short distance above
the tube the distal extremity of the columella auris may be observed
as a dense cellular mass, which posteriorly grades into true car-
tilage.

Stage XIV.—Young toad, about 9 mm (figs. 30, 31).

In this stage the tympano-Eustachian passage has the same
general position and relations that distinguish it in the fully mature
animal. Relatively it is not so large as in the latter, nor is its
lumen complete throughout, but in all other respects it is essentially

like the adult structure.   Figs. 30 and 31 illustrate the condition
of the tube at this time.   In fig. 30 we have a transverse section
through one side of the head immediately back of the eye.   To the
outer side is shown the quadrate cartilage, which in the present
stage stands almost vertical and hence is shown in the figure cut
throughout the greater part of its length.   External to the upper
portion of the cartilage is the tympanic portion of the Eustachian
tube, showing a slight lumen (*Tym.*).   I have another specimen of
approximately the same age in which the lumen is much larger,
forming a considerable cavity.   Underlying this portion is the
tympanic or annular cartilage, which in its ventral portion, at
least, is completely chondrified (*An.*).   The fully formed car-
tilage does not, however, form a complete ring.   Internally the
tube approaches the outer surface of the quadrate, as was the case
in the earlier stages.   Applied to the dorso-external wall of the
tympanic cavity is the distal extremity of the columella auris, at
present a very compact cellular mass, not yet differentiated into
true cartilage (*Cl.*).   The apparent inclusion of the columella
within the tympanic cavity is produced by the subsequent growth
of the latter around this portion of the cartilage.   Attached to
the ventral surface of the annular cartilage are fibres of the
depressor ossis hyoidei (*m.d.h.*).   The attachment of the muscle
to the cartilage was acquired soon after the earliest *Anlage* of the
latter had appeared.   At its ventral end the muscle has lost its
attachment to the hyoid cartilage and has acquired a new insertion
into the angle of the mandible, so that like the depressor man-
dibulæ it serves to depress the latter (compare also fig. 31).   The
bulk of the muscle lies posterior to the Eustachian tube.   Internal
to the muscle and between it and the quadrate are two blood-
vessels, which correspond to the original mandibular aorta (*m.a.*).
This vessel, as we have seen, was an important one during the
tadpole period, but during the metamorphosis it underwent some
profound changes.   Its middle portion largely degenerated, so
that the vessel became divided into a proximal and a distal half.
The vessel undergoes other changes, but these I have not been able
to follow satisfactorily with the material at hand.

Fig. 31 shows a section through the tympano-Eustachian tube
near its posterior boundary.   The quadrate (*q.*) is here seen in
two separate portions, a dorsal and a ventral.   This condition can

be readily understood by referring to the description of the cartilage as given in stage XIII. It suffices to mention that the section passes back of the point where the quadrate curves backward on itself, so that the dorsal is the proximal, the ventral the distal portion of the cartilage. The distal portion bears the mandible. Underlying the proximal portion is the Eustachian tube (*Eu.*), here shown in three detached segments. Other sections, however, show these segments continuous, so that the tube is now complete. Moreover, the proximal innermost segment is continuous with the pharynx and in reality represents a diverticulum (*div.*) from the latter. In the other specimen that I have of this stage this portion is continuous with the pharynx, but its distal extremity ends blindly without forming a connection with the Eustachian tube. In the toad of stage XIII this diverticulum of the pharynx was also present, and connecting it with the widely separated tubal *Anlage* was a dense strand of connective tissue cells, whose long diameters were extended in a direction coinciding with a line drawn between the separated parts. By means of this diverticulum the tympano-Eustachian tube is now united to the pharynx. The tube presents throughout an irregular lumen, bounded by a well-defined columnar epithelium. That portion of the tube which is most externally situated is the posterior part of the tympanic cavity (*Tym.*). Attached to the dorsal wall of the latter is the columella auris (*Cl.*). The ramus hyomandibularis of the facial nerve is not shown in this section, since, owing to the posterior flexure of the quadrate and the separation of the hyoid from the latter, the nerve now lies entirely posterior to the tube.

SUMMARY.

The results recorded in the preceding pages may be briefly summarized as follows:

1. The tympano-Eustachian passage is in the main derived from the dorsalmost portion of the hyomandibular fold (cleft).

2. In the earliest stages described, the hyomandibular fold is present as a solid, plate-like fold extending outward and forward beneath the eye region and terminating laterally in a free edge situated a short distance below the ectoderm. Its attachment to the ectoderm is lost at about this stage.

3. At first the outer or distal edge of the hyomandibular fold
is smooth and unbroken throughout its entire extent.   Later, this
edge becomes interrupted in its middle portion by the formation of
a progressively deepening depression, which ultimately reaches the
pharyngeal wall and divides the hyomandibular fold into two
parts—a dorsal cord-like portion, the future tympano-Eustachian
passage, and a ventral portion forming a shallow sacculation to
the ventro-lateral portion of the pharyngeal cavity.

4. The ventral portion of the hyomandibular fold ceases to be
recognizable after the late tadpole stages.   It is this portion which
Villy considers as the last remnant of the hyomandibular fold.

5. The earliest evidence of the degeneration of the hyoman-
dibular fold is afforded by the recession of its outer edge from the
neighborhood of the external ectoderm.   Only the dorsalmost
portion of the fold continues in intimate proximity to the skin.
The withdrawal of the remainder is associated with (1) the reduc-
tion in size of the pharynx, in consequence of the segregation of
the surrounding mesenchyme to form the *Anlagen* of muscles and
cartilages, and (2) the development of the muscles of the hyoid
arch—the depressor mandibulæ and ossis hyoidei.   Of these mus-
cles the depressor mandibulæ extends forward between the skin
and the outer border of the hyomandibular fold and acquires
attachment to the developing quadrate cartilage in front of the
fold.   It thus interposes an effective barrier to further outward
extension of the fold.   Only the dorsalmost portion of the fold
remains unimpeded by the muscle, and this accordingly retains its
proximity to the ectoderm and in the subsequent growth of the
head is carried outward as a narrow, cord-like strand expanded at
its outer extremity into a club-shaped swelling.   This portion I
have designated the " diverticulum."   It is the *Anlage* of the
tympano-Eustachian passage.

6. The outer hyoidean muscle, the depressor ossis hyoidei, also
acquires attachment to the quadrate *Anlage* at a point above and
posterior to the hyomandibular cleft.   The " diverticulum " or
Anlage of the tympano-Eustachian passage thus comes to lie
between the two hyoidean muscles.

7. The growth anteriorly of the hyoidean muscles produces a
marked antero-posterior extension of the hyomandibular fold and
of its derivative, the tympano-Eustachian *Anlage*.   This antero-

posterior direction taken by the tubal *Anlage* is characteristic of it during the entire larval period.

8. The further degeneration of the hyomandibular fold is correlated with the subsequent increase in size of the muscles already mentioned, the union of the hyoid cartilage with the quadrate and the enlargement of the mandibular aortic arch.

9. After the degeneration of the hyomandibular fold the *Anlage* of the tympano-Eustachian passage persists as a minute, solid cord, extending along the outer surface of the processus muscularis of the quadrate. Posteriorly it is attached to the wall of the pharynx at a point posterior to the quadrato-hyoid articulation. Anteriorly and distally it expands to form the club-shaped *Anlage* of the tympanic cavity.

10. During the active tadpole period the tympano-Eustachian *Anlage* undergoes marked degeneration. This degeneration is confined to the middle and posterior parts of the *Anlage*, the distal expanded portion retaining its original relative size throughout the entire larval period. The degeneration is in all probability connected with the growth of the two muscles—depressor mandibulæ and ossis hyoidei—between which it lies. Owing to the lack of space it is unable to keep pace with the surrounding structures in the subsequent growth of the animal.

11. In the early tadpole period the tympano-Eustachian *Anlage* is continuous posteriorly with the wall of the pharynx. Later the connection between the two apparently disappears, though the time of its disappearance seems to vary in different individuals. An indistinct strand may continue to unite the two parts, but this I have been unable to demonstrate.

12. The degeneration of the tympano-Eustachian *Anlage* is carried to an extreme in the later tadpole stages. At this time it is apparently broken up into a number of fragments of varying length. This fragmentation is probably more apparent than real, being produced by the restriction of the more vital stainable substance of the tubal *Anlage* to areas less subject to the pressure of the neighboring structures. The irregular distribution of the fragments, both in different individuals and on different sides of the same individual, favors the view that a compressed, transparent cord still connects the apparently separate parts. In one specimen

17

(tadpole of about 18 mm.) I have been enabled to trace out such a connecting cord.

13. Regeneration of the tympano-Eustachian *Anlage* begins at a period immediately preceding the period when the fore-limbs break out of the opercular cavity.

14. The later metamorphosis of the tubal *Anlage* is connected with the modifications of the neighboring skeletal structures, particularly with the posterior rotation of the quadrate. By this means the tubal cord comes into relation with the auditory region of the skull and the various fragments are brought closer together, so that they can readily unite.

15. The acquisition of a lumen by the tubal *Anlage* takes place gradually, beginning at the close of the metamorphosis. Details apparently vary in different individuals.

16. Completion of the tympano-Eustachian passage is effected by an outgrowth from the pharynx which unites with the tubal *Anlage*.

17. The final position of the tympano-Eustachian tube between the mandibular and hyoid bars is produced by the separation of the latter from the quadrate and its attachment to the auditory capsule posterior to the tube.

18. The annular cartilage arises at a stage immediately preceding the protrusion of the fore-limbs. Its *Anlage* forms a dense cellular strand derived from the perichondrium of the quadrate and surrounding the tympanic portion of the tubal *Anlage*. It does not begin to form fully differentiated cartilage until after the close of the metamorphosis.

19. The stapes arises within the membrane closing the fenestra ovalis. It has no connection with any of the visceral-arches.

20. The columella auris is first met with in the early stages of the metamorphosis, as a compact cellular strand extending forward from the stapes and terminating imperceptibly in the connective tissue. It continues to grow forward and acquires connection with the quadrate. Continued growth brings it in contact with the tympanic cavity. Chondrification begins in the posterior portion of the rod.

EXPLANATION OF PLATES VI, VII, VIII, IX.

The drawings were outlined by aid of the camera lucida, and with the exception of figures 26 and 29 were all drawn to the same scale. With the exception of the two mentioned they are also slightly diagramatic—those on plates VI–VIII reduced one-third ; on plate IX, one-half.

REFERENCE LETTERS.

An.—Annular cartilage.
Aud.—Auditory capsule.
Car.—Carotid artery.
Ch.—Chorda.
Cl.—Columella auris.
div.—Diverticulum from pharynx.
E.—Eye.
Eu.—Eustachian cord (or tube).
Ext.—External gill.
H.—Hyoid cartilage.
h.a.—Hyoidean aortic arch.
h.m.—Hyoidean muscle mass.
Hym.—Hyomandibular fold.
inf.—Infundibulum.
k.m.—Muscles of mastication.
l.r.—Lateral recess of pharynx.
M.—Meckel's cartilage.
m.a.—Mandibular aortic arch.
m.a'.—Its efferent portion.
m.a''.—Its afferent portion.
m.d.h.—Depressor hyoidei.
m.d.m.—Depressor mandibulæ.
m.d.m'.—Its accessory slip.
Ol.—Olfactory depression.

Pr.A.—Processus ascendens.
Pr.q.c.a. — Commissura quadrato-cranialis anterior.
Pr.M.—Processus muscularis.
Q.—Quadrate.
St.—Stomatodeal plate.
Th.—Thyroid.
Tr.—Trabecula cranii.
Tym.—Tympanic (distal) portion of Eustachian cord.
X.—Small blood-vessel connecting mandibular and hyoid aortic arches.
Y.—Small blood-vessel external to ramus hyomandibularis.
2, 3, 4, 5, v.f.—Second, third, fourth and fifth visceral-cletts.
V.—Trigeminal ganglion.
V'.—Ophthalmic ganglion.
V''.—Maxillary ganglion.
V.m.—Maxillo-mandibular nerve.
VII.—Facial ganglion.
vii h.—Ramus hyomandibularis.
vii pl.—Ramus palatinus.

PLATE VI, Fig. 1.—Coronal section through the pharynx and visceral-clefts of an embryo of stage I.

Fig. 2.—Coronal section through the same region of a slightly older embryo (stage II).

Fig. 3.—Coronal section of the same embryo at a somewhat higher plane.

Fig. 4.—Coronal section through the dorsalmost portion of the pharynx of the same embryo.

Fig. 5.—Transverse section through the head of an embryo of approximately the same stage as the last. The section on the right side passes through the extreme anterior portion of the hyomandibular fold (*Hym.*). The plane of section is considerably farther posterior on the left side.

Fig. 6.—Transverse section of the head of the same embryo. On the right side the hyomandibular fold is cut throughout the greater part of its length (*Hym.*). The dorsalmost portion of its outer (distal) border approaches most closely the skin. On the left the facial ganglion gives off the ramus hyomandibularis just external to the outer end of the fold (*vii h.*).

PLATE VII, Fig. 7.—Transverse section of the head of the same embryo slightly posterior to the last.

Fig. 8.—Coronal section of head of tadpole of stage V. One side alone shown.

Fig. 10.—Transverse section of the head of a young tadpole of stage III. The section is through the anterior end of the pharynx. The plane of section is more posterior on the right side than on the left.

Fig. 11.—Fourth section posterior to that of figure 10. *Eu.* designates the "diverticulum," while at *Hym.* is the antero-inferior portion of the hyomandibular fold.

Fig. 12.—Third section posterior to the last. The diverticulum (*Eu.*) is now continuous with the antero-inferior portion (*Hym.*) of the hyomandibular fold. Between the two is the depression lodging the muscles (*m.d.m.* and *m.d.h.*). The small vessel above *Eu.* is the mandibular aortic arch.

Fig. 13.—Sixth section posterior to the last. The mandibular aortic arch is dorsal to *Hym.*

PLATE VIII, Fig. 14.—Third section posterior to last. The mandibular aortic arch on the right side is just internal to *vii pl.*

Fig. 15.—Transverse section of head of tadpole of stage IV in the region immediately posterior to that shown in figure 24.

Fig. 16.—Coronal section of the head of a young tadpole of stage II. On the right side the section passes a slight distance above the floor of the pharynx, while on the left it is considerably higher. The small vessel in front of *Hym.* is the mandibular aortic arch.

Fig. 17.—Fourth section dorsal to the last. On the right side the little protrusion of the pharyngeal wall just internal to *Tym.* is the antero-inferior portion of the hyomandibular fold just below the point where it becomes continuous with *Tym.* The space between the two is the depression.

Fig. 18.—Coronal section of the head of the same animal a slight distance below the roof of the mouth.

Fig. 19.—Transverse section of the head of a tadpole of stage IV in the region of the processus ascendens.

PLATE IX, Fig. 23.—Transverse section of the head of a tadpole of stage IV, passing through a region slightly anterior to the auditory sac.

Fig. 24.—Transverse section of the head of a tadpole of stage IV, showing the anterior expanded portion (*Tym.*) of the Eustachian cord. *Hym.* denotes the proximal anterior portion of the hyomandibular cleft.

Fig. 25.—Transverse section of the head of a tadpole of stage V. On the left the section is immediately posterior to the eye. The minute upgrowth from the dorsal wall of *l.r.* just internal to *vii h.* is the Eustachian proliferation.

Fig. 26.—Transverse section of a portion of the right side of the head of an old tadpole of 18 mm. (stage VI). This section is considerably more magnified than the others and is intended to show the extremely rudimentary character of the Eustachian cord at this stage.

Fig. 28.—Transverse section of the right side of the head of a tadpole at the beginning of the metamorphosis (stage VIII), showing the formation of the annular cartilage.

Fig. 29.—Transverse section of a portion of the right side of the head of the same animal. The section was drawn with the same degree of magnification as figure 26 and is intended to show the Eustachian cord when it begins to regenerate.

Fig. 30.—Transverse section through one side of the head of a young toad (stage XV). The section passes through the region immediately in front of the ear-capsule.

Fig. 31.—Transverse section through one side of the head of the same animal. The section passes through the anterior portion of the ear-capsule.